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CITATION:

WADA, JURO ...[et al]. The Experimental Study on the Transbronchial Administration of Carcinostatic Agents in Pulmonary Carcinoma : Preliminary Report. 日本外科宝函 1968, 37(2): 272-278

ISSUE DATE:

1968-03-01

URL:

<http://hdl.handle.net/2433/207453>

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The Experimental Study on the Transbronchial Administration of Carcinostatic Agents in Pulmonary Carcinoma

— Preliminary Report —

by

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Received for Publication Jan. 10, 1968

In spite of various progressive therapeutic means, postoperative long term cure rate of pulmonary cancer is mainly influenced by lymphatic metastasis. However, attempts for selective administration of agents for pulmonary and mediastinal lymphatic system have not been reported. Therefore, in the present paper basic experiments on transbronchial administration of carcinostatica or radioisotope therapeutic agents are reported.

METHOD

Mongrel dogs anesthetized with pentobarbital sodium and intubated for artificial respiration were used. The angulus venosus were exposed on both side of the neck and retrograde thoracic duct cannulations using polyethylene tubes were made at the sites where bilateral thoracic duct empty into the vein. Polyethylene tubes with an inner diameter of 0.7-1.1 mm were used and coated with heparin to prevent coagulation of lymph fluid.

The left thoracic duct was ligated at the venous side and a thin polyethylene tube coated with heparin was inserted retrogradely for the left thoracic duct lymph collection. The right thoracic duct usually consist of numerous narrow branches making cannulation difficult. Therefore, the tube was inserted into the vein directly above the flow-in site as shown in Fig. 1 to prevent mixing of the venous blood with lymph to be collected.

Two methods of transbronchial administration of agents, inhalation and instillation were employed. The former was conducted by I.P.P.B. nebulization. After agent administration, thoracic duct lymph samples were collected and blood samples were taken from a femoral artery. The dogs were later sacrificed for parenchymatic tissue studies of intra-thoracic lymph nodes, lung, liver, spleen, kidney and bone marrow.

As the agents P^{32} -Thio-TEPA, Co^{60} -Protoporphyrin, Au^{198} colloidal solution and

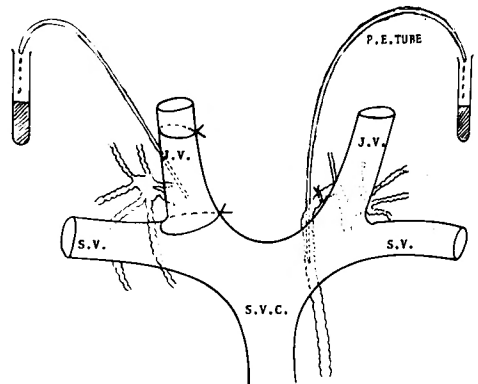


Fig. 1 Method for Collection of the Right and Left Thoracic duct Lymph.

P^{32} phosphate solution were used, the radioactivity of the lymph and blood samples were assayed of the beta activity for P^{32} and respective gamma activity for Co^{60} and Au^{198} measured with Geiger-Muller counter and well type scintillation counter. They were expressed by counts/min./ml. The tissue sample of organs taken for the beta activity measurement were washed thoroughly with water, the weight was measured, and after mincing this was digested by HNO_3 . This was further homogenized and dry ashed and counts/min./g was measured. For the measurement of gamma activity, instead of dry ashing, the homogenate was diluted in 10 ml and measured with a well type scintillation counter. By the assay the back counts were decreased and corrected for natural decay up to time of the assay from administration.

RESULTS

1) P^{32} -labelled sodium phosphate saline solution.

In three dogs after the $600\mu c$ of P^{32} solution was instilled positive pressure breathing was maintained for one hour. In two dogs the solution was given by nebulization for 30 minutes. The assay values were as shown in Fig. 2, higher in the lymph of right thoracic duct, the left duct and in the blood in this order.

Concentration of P^{32} in various organs 3 to 10 days after the experiment were evenly high indicating a considerable leakage of the radioactivity from the lung to other organs such as liver, bone marrow and kidneys via the blood stream. There were no difference between the instillation and nebulization group. (Table 1)

2) Au^{198} colloidal solution.

2 mc of Au^{198} was administered by instillation to each of three dogs and by nebu-

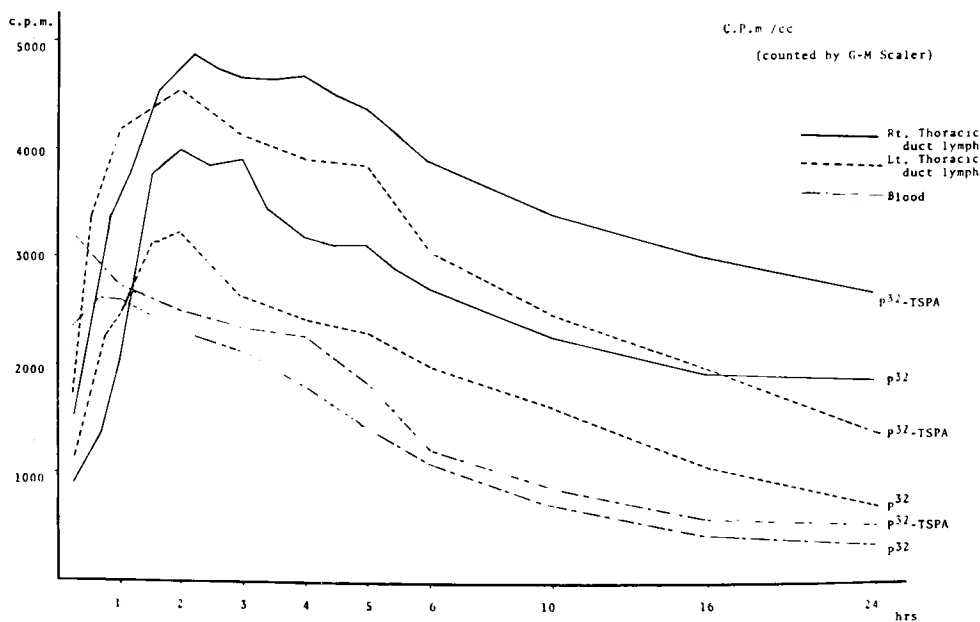


Fig. 2 Radioactivity of Blood & Lymph after Transbronchial Administration of P^{32} labeled Agents. ($600\mu c$)

Table 1 Tissue Distribution of Radioactivity 3 Days after Transbronchial Administration of RI labeled Agents in Dogs.

Agent	Counts/min./g			
	P ³²	Au ¹⁹⁸	P ³² -TSPA	CO ⁶⁰ -COPP
	600μc Inhalation	2mc Inhalation	600μc Inhalation	4μc Instillation to L. low. lobe Br.
L. upper mediast. L.N.	144	23,125	449	3,088
R. upper mediast. L.N.	274	65,221	560	30,066
L. paratracheal L.N.	150	—	—	63,350
R. paratracheal L.N.	720	33,450	450	19,350
Botallow's L.N.	960	95,921	788	82,108
Subcarinal L.N.	1,448	113,543	1,800	4,234
L. bronchial L.N.	1,272	45,326	380	—
R. bronchial L.N.	1,162	83,296	496	0
R. upper lobe	356	2,456	435	320
R. middle lobe	274	3,168	557	21
R. cardiac lobe	223	2,763	550	0
R. lower lobe	347	4,865	335	50
L. upper lobe	216	1,962	535	570,910
L. lingula	198	1,118	411	12,131
L. lower lobe	276	2,563	408	160
Liver	1,352	865	1,573	767
Spleen	584	462	902	148
Kidney	1,025	3,569	1,602	533
Bone marrow	1,450	459	737	8

— no specimen 0 below background

c.p.m./cc
(counted by Scintillation Counter)

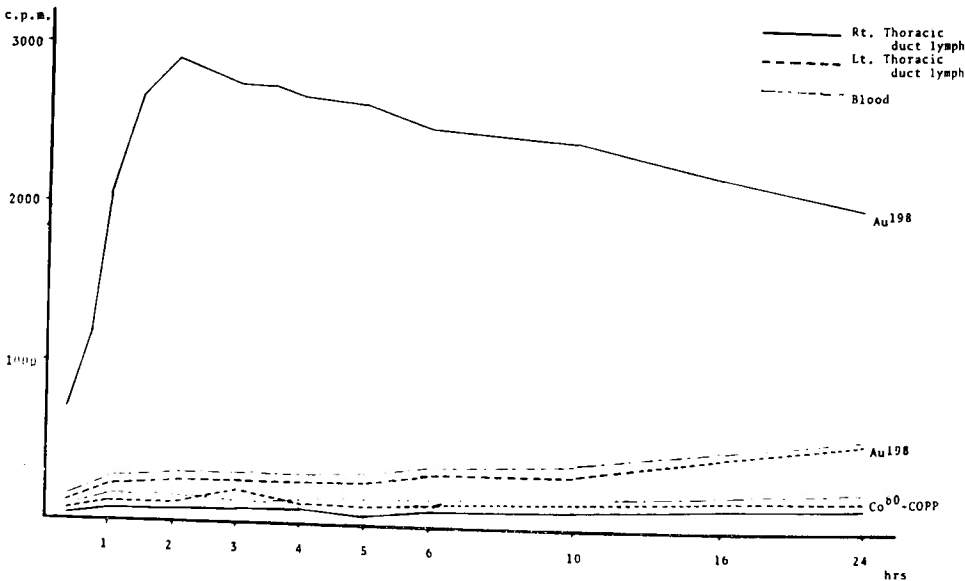


Fig. 3 Radioactivity of Blood & Lymph after Transbronchial Administration of Au¹⁹⁸ Colloid (2 mc) and Co⁶⁰-COPP (4μc).

lization to three dogs. The results of radioactivity assay were summarized in Fig. 3 and Table 1. The radioactivity appeared in the right thoracic duct lymph at an early period, specifically and in a large amount, and showed a lasting high concentration. In the left thoracic duct lymph and in the blood, concentration showed extremely low value at the beginning and thereafter a gradual increase were seen. Radioactivity assay study revealed as shown in Table 1 considerable selectivity of Au^{198} to 5 days after the administration showed a specific high concentration in the hilar and the mediastinal lymph nodes, a slightly high in the lung parenchym and low in other organs excluding the kidney.

3) P^{32} -TSPA (Triethyrene Thio-phosphoramide)*

P^{32} -TSPA, corresponding to $600\ \mu\text{c}$ of P^{32} and 20 mg. of Thio-TEPA, was made into a polyethylene glycol solution to which 3 ml. saline solution was added. This was administered transbronchially into 3 dogs by instillation while remaining 3 dogs were administered with the nebulized inhalation method.

Periodical assays of radioactivity in lymph and blood were summarized in Fig. 2 and Table 1. The distribution in the lymph nodes and organ tissue at 3 to 10 days after transbronchial administration showed no selective absorption by the pulmonary lymphatic system, and a considerable distribution in the liver, spleen, kidneys and bone marrow was observed.

4) Co^{60} -Protoporphyrin (Co^{60} -COPP)**

Co^{60} -COPP powder was dissolved in a pH 7.4 phosphate buffer solution and was adjusted in such a way as to contain $4\ \mu\text{c}$ of Co^{60} or 2 mg of COPP in 4 cc of the solution. $4\ \mu\text{c}$ of Co^{60} -COPP was instilled into the lobar bronchus of 3 dogs. The gamma activity in the lymph and blood was assayed. Results showed low activity in both lymph and blood as shown in Fig. 3. However, at 1 to 5 days after the instillation, the distribution in the lymph nodes and organ tissue shows a high concentration of radioactivity in the hilar and the mediastinal lymph nodes together with a high concentration of radioactivity in the parenchym of the instilled lobe. In contrast, in other lobes and other organs hardly any radioactivity was seen with a small amount in the liver. (Table 1) The high activity measured in the instilled lobe in early stage gradually disappeared with the lapse of time and the activity was minimum at 5 days after instillation.

DISCUSSION

In the present paper the authors, with the intent of preventing lymphatic metastasis which has a strong bearing in postoperative lung cancer, attempted to administer a high concentration of carcinostatic agents selectively into the pulmonary and mediastinal lymph nodes transbronchially. To determine the distribution of the agents in the pulmonary lymphatic system, thoracic duct lymph fluid was collected by bilateral thoracic duct cannulations⁶⁾¹⁸⁾ and the radioactivity in the pulmonary and mediastinal lymph nodes were assayed. These were compared against the concentration in the blood and other organs.

In the case of P^{32} tracer, when such a low molecular weight substance is given in the form of solution, a considerable amount passes through the alveolar wall capillary and

* Synthesized by the Sumitomo Atomic Energy Kogyo Co., Takarazuka, Japan.

** Synthesized by Japan Blood Bank Co., Tokyo, Japan.

is directly absorbed by the blood. Thus, there is no essential difference between intravenous administration except that the former method shows a higher distribution in the pulmonary field.

Concerning Au^{198} , colloidal solution administered transbronchially, experiments have already been done by MENEELY¹¹⁾, HAHN⁹⁾ and BERG²⁾ in 1951 for the purpose of local irradiation. As compared with P^{32} , Au^{198} has a higher molecular weight, a larger particle size and further because it is a colloidal solution, transfer into the blood was limited and moreover since Au^{198} has an affinity to the reticuloendothelial system and to the lymphatic system, in our present work it was reascertained that very high concentration was seen in the pulmonary lymph fluid and lymph nodes. Among known carcinostatica with special regards to P^{32} -TSPA and Co^{60} -COPP used in our present work, the distribution in the body after administration was traced respectively by their radioactivity. It was impossible to obtain an accurate P^{32} -TSPA distribution in the body because the analysis of the assayed P^{32} radioactivity was not done whether it came from liberated P^{32} or from P^{32} -TSPA itself. However, the approximate trend of distribution was almost the same as that of P^{32} phosphate solution. The molecular weight was 189.22 and in a saline solution the leakage into the blood was considerable rendering it impossible for local administration.

The molecular weight of Co^{60} -COPP is 620, and at the time of purification the original powder had a particle distribution size of 1 to 200 μ or thereabouts. In a solution form since it could not pass a cellophane membrane it may be surmized that Co^{60} -COPP instead of passing the alveolar wall capillaries, is transferred via the bronchial endothel and is entrapped by the bronchial wall lymphatic system from which it passes into the lymph flow and is settled in the hilar and mediastinal lymph nodes. Thus, these tissue alone show a high intensity resulting in a minimal leakage to the entire body. From the above results, it seems that the particle size of the agents to be administered is the first condition to be met in order to have the agent selectively absorbed by the pulmonary and mediastinal lymph nodes after transbronchial administration, which is our primary objective.

Absorbing effect of various substances by normal lungs was showed experimentally by many authors³⁾¹³⁾¹⁴⁾ and it was shown that the route of absorption differs with the individual size of the particles⁴⁾⁸⁾¹²⁾¹⁷⁾¹⁰⁾.

(Fig. 4) So the particle size will be the point of discussion. ABRANSON¹⁾ and TAPLIN¹⁵⁾ (1950) reported the relation between particle size and the depth of its reach in the lungs. A limit in particle size for the absorption by the bronchial wall was also reported by GILLIN⁷⁾ (1938), DRINKER⁵⁾ (1947) and others.

To make particle size even, TAPLIN et al¹⁶⁾ (1951) tagged *B. subtilis* spores with P^{32} and conducted experiments. With the same purpose we have used various agents

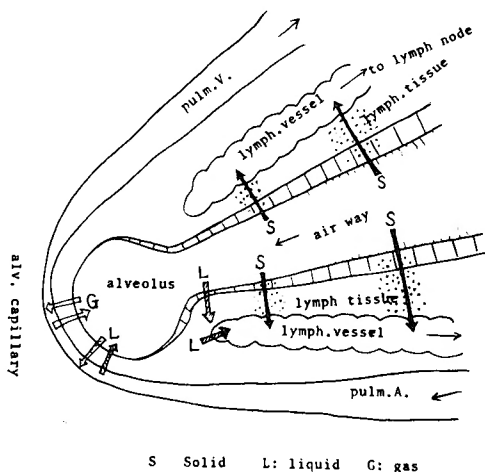


Fig. 4 Absorption of intrabronchial substance.

adsorbed by ion exchanger resin which was smashed and filtered in particle size of 0.5 to 5 μ . These experimental work is underway at present and will be published later.

From the experiments we have conducted we can prospectively summarize the conditions to be met in the clinical application of transbronchial administration of carcinostatica are : (1) The agent must have a uniform distribution size of 0.5-2 μ or thereabouts. Well soluble agents should be adsorbed by solid fine particles of this size and used. In the case of larger size particles, they are used in suspension form, (2) the nebulizer apparatus should deliver a narrow range of size distribution, (3) the agent at the desired therapeutic dose, should be non irritating to the mucous membrane of the bronchus, and (4) the agent should have a strong cartinostatic action and should be retained by the local lymph node for a considerable length of time and also have a long lasting effect.

SUMMARY

Using adult mongrel dogs P^{32} , Au^{198} colloidal solution, P^{32} -Thio-TEPA and Co^{60} -COPP were administered transbronchially by inhalation or instillation and an assay was conducted on the radioactive intensity of each agent in the right and left thoracic duct lymph, blood, hilar and mediastinal lymph nodes and various other organs. The distribution of the agents in the body after administration was investigated.

1) In P^{32} and P^{32} -TSPA which has a small particle size, a large amount was absorbed into the blood through the alveolar wall capillaries and selective administration to the pulmonary lymphatic system was not achieved.

2) In Au^{198} administration, because of its lymphatic affinity and because it is a colloidal solution, the agent appeared specifically and at a high concentration in the pulmonary lymphatic system.

3) Since the particle size of Co^{60} -COPP is large the absorbing into the blood via the alveolar capillaries was extremely small and through the bronchial endothel a high distribution into the hilar and mediastinal lymph nodes was seen.

4) Based on these facts, it was suggested that it would be possible to selectively administer carcinostatic agents with an even particle size distribution transbronchially to the pulmonary and mediastinal lymph nodes of clinical lung cancer patients. And same suggestions were discussed.

5) Any possible side effects of the agents on the respiratory organs, together with the clinical indications for this type of administration has to be evaluated.

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和文抄録

肺癌転移に対する抗癌剤経気道投与の実験的研究

(第 1 報)

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肺癌患者に対する外科的根治手術の遠隔成績を大きく左右する因子として、肺門及び縦隔リンパ節転移があげられるが、われわれはこれらリンパ節転移を防止する目的で抗癌剤の経気道的投与を試み実験的研究を行なった。

雑種成犬を用いて静脈麻酔下に気管内挿管より P^{32} 生食溶液、 Au^{198} 金コロイド溶液、 P^{32} Thio-TEPA、 Co^{60} -COPP などを経気道的に吸入又は注入により投

与し、それらの濃度分布を両側胸管リンパ液、血液、胸腔内諸リンパ節及び肺、肝、脾、腎など実質臓器中の放射能測定により検索した。

結果は Au^{198} および Co^{60} -COPP で肺門、縦隔リンパ系への選択的吸収がみられ、これより投与する薬剤の粒子条件を考慮して、均一な微粒子として投与するならば、これら抗癌剤の肺、縦隔リンパ系への選択的投与が可能であることが分つた。